METHOD FOR THE QUANTITATIVE DETERMINATION OF D-3-HYDROXYBUTYRIC ACID AND ACETOACETIC ACID, AND ANALYTICAL REAGENT THEREFOR

TECHNICAL FIELD

The present invention relates to a method for the quantitative determination of D-3-hydroxybutyric acid and acetoacetic acid utilizing an enzymatic cycling reaction. The present invention also relates to a novel analytical reagent for use in the above-mentioned quantitative determination.

BACKGROUND ART

In clinical examination, it is important to determine ketone bodies, such as D-3-hydroxybutyric acid and acetoacetic acid, as criteria for detecting metabolic failure. In addition to the above-mentioned two types of ketone bodies, acetone can also be mentioned as a ketone body. However, acetone is volatile and unstable. In addition, the concentration of acetone in blood is considerably low as compared to the concentrations of D-3-hydroxybutyric acid and acetoacetic acid. This means that a metabolic failure can be successfully detected even by determining only D-3-hydroxybutyric acid and acetoacetic acid among ketone bodies.

Examples of conventional methods for the determination of ketone bodies include the diazonium method in which acetoacetic acid is reacted with a diazonium salt to thereby produce a hydrazo compound or an azo compound, and the absorbance of the produced hydrazo or azo compound is measured; the nitroprusside method in which acetoacetic acid and acetone are reacted with a nitroprusside reagent to thereby convert acetoacetic acid and acetone to respective colorimetrically-detectable forms; the gas chromatographic method in which D-3-hydroxybutyric acid and acetoacetic acid are converted to acetone and all of the ketone bodies are quantitatively determined in terms of acetone by gas chromatography; and the enzymatic method.

Although the diazonium method exhibits high sensitivity, the method requires deprotenization of a biological sample before conducting the reaction of acetoacetic acid with a diazonium salt. Further, for determining D-3-hydroxybutyric acid by the diazonium method, the following cumbersome operations are required. Acetoacetic acid in a biological sample is determined and then, D-3-hydroxybutyric acid in the sample is converted to acetoacetic acid by D-3-hydroxybutyrate dehydrogenase, followed by measurement of the acetoacetic acid (which is converted from the D-3-hydroxybutyric acid), thus quantitatively determining both of the D-3-hydroxybutyric acid and acetoacetic acid in the sample. Then, the quantity of D-3-hydroxybutyric acid is obtained by subtracting the quantity of acetoacetic acid from the above-mentioned total quantity of D-3-hydroxybutyric acid and acetoacetic acid. However, the conventional methods in which a ketone body is quantitatively determined by reacting the ketone body with a chemical reagent generally has low specificity and, therefore, the determination of a target substance is likely to be influenced by other substances present in a biological sample. For example, in the diazonium method, the determination of acetoacetic acid in a biological sample is likely to be influenced by oxaloacetic acid (Clinica Chemica Acta, vol. 134, p.327–336, 1983). With respect to the nitroprussid method, this method has disadvantages in that not only cannot D-3-hydroxybutyric acid be directly detected as in the case of the diazonium method, but also the method exhibits low sensitivity (detection sensitivity: 500 to 1000 μM) (Extra-edition of Japanese Journal of Clinical Medicine vol, 47, p.484, 1989).

Further, the gas chromatographic method is extremely cumbersome and, therefore, the method is not suitable for use in clinical examination in which a lot of biological samples are to be tested.

With respect to the enzymatic method, there can be mentioned Williamson method (Method of Enzymatic Analysis, Academic Press, New York, p.1836–1843, 1974) in which the enzymatic reaction of D-3-hydroxybutyrate dehydrogenase (EC 1. 1. 1. 30) is utilized. In this method, when acetoacetic acid in a biological sample is to be determined, the reverse enzymatic reaction under the action of D-3-hydroxybutyrate dehydrogenase is utilized in which acetoacetic acid and a reduced form of nicotinamide adenine dinucleotide (NAD) are, respectively, converted to D-3-hydroxybutyric acid and NAD. That is, a decrease in the amount of reduced NAD (which decrease corresponds to the amount of reduced NAD consumed in the above reverse reaction) which decrease is caused for a predetermined period of time is measured. On the other hand, when D-3-hydroxybutyric acid in a biological sample is to be determined, the forward enzymatic reaction under the action of D-3-hydroxybutyrate dehydrogenase is utilized in which D-3-hydroxybutyric acid and NAD are, respectively, converted to acetoacetic acid and reduced NAD. That is, an increase in the amount of reduced NAD (which increase corresponds to the amount of reduced NAD produced in the above forward reaction) which increase is caused for a predetermined period of time is measured. A modification of the Williamson method was also proposed.

As another example of the enzymatic method, there can be mentioned a paper strip testing for the determination of D-3-hydroxybutyric acid. In this method, the forward enzymatic reaction under the action of D-3-hydroxybutyrate dehydrogenase is utilized in which D-3-hydroxybutyric acid and NAD are, respectively, converted to acetoacetic acid and reduced NAD under the action of D-3-hydroxybutyrate dehydrogenase. An increase in the amount of reduced NAD corresponds to the amount of reduced NAD produced in the above forward enzymatic reaction. The amount of the D-3-hydroxybutyric acid can be determined based on the increase in the amount of reduced NAD. The reduced NAD obtained in the above enzymatic reaction is reacted with a tetrazolium salt on a strip paper, and then a formazan dye produced in the amount which is proportional to the increase in the amount of the reduced NAD is determined.

In the above-mentioned enzymatic methods, it is impossible not only to determine both of D-3-hydroxybutyric acid and acetoacetic acid by a single enzymatic reaction, which are clinically important ketone bodies, but also to achieve the determination with high sensitivity.

As still another example of the enzymatic method for the quantitative determination of acetoacetic acid, there can be mentioned a method in which acetoacetic acid is converted to acetyl-CoA by using acetoacetyl-CoA synthetase (EC 6.2.1.16) and 3-ketoacyl-CoA thiolase (EC 2.3.1.16) and then, the acetyl-CoA produced is used for acetylating aniline under the action of arylamine acetylase (EC 2.3.1.5), followed by measurement of a decrease in the amount of acetylated aniline as a decrease in the absorbance at a wavelength of 405 nm (Acta Biochim. Biophys. Acad. Sci. Hung., vol. 7, p.143, 1972). However, this method requires complicated operations. In addition, a highly sensitive